Herd-level risk factors for the introduction and spread of *Salmonella* in pig herds.

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Introduction

Salmonella has been identified in all stages of pork production. Efforts to decrease the *Salmonella* burden on society should be targeted at all levels of the production chain. One of the greatest challenges for *Salmonella* free or controlled pork production lies in identifying effective measures that can be taken at the herd-level.

The study presented here, sets out to identify herd factors associated with (sub-clinical) *Salmonella* infection in pigs, such as management routines, feeding strategies and bio-security procedures. The investigation was part of an international research programme, mainly sponsored by the European Commission, entitled '*Salmonella* in Pork' (1). A rather unique property of this study was that the same protocol was used in Denmark, Germany, Greece, the Netherlands and Sweden, allowing for direct comparison between countries and over-all modelling of *Salmonella* epidemiology. As country-specific risk factor analyses are presented elsewhere (2, 3), this paper describes the over-all analysis of risk factors for sub-clinical *Salmonella* infection in finishing pig herds in the participating EU-member states.

Materials and Methods

Data collection

A standard risk-factor questionnaire was developed, comprising data on herd size and type, management practice, health problems observed, use of antibiotics, method of introduction of pigs (breeding animals, feeders), hygiene regime, and feeding strategy. After consensus on the contents of the questionnaire was achieved among the participating researchers, a data entry program was developed and distributed, to ensure standardised data recording and data management.

In each of the participating countries, being Denmark, Germany, Greece, the Netherlands and Sweden, at least 60 herds with finishing pigs were selected. In each herd, 50 blood samples were taken from animals close to slaughter. Blood samples collected in the Netherlands were analysed with an indirect mix-ELISA, developed in the Netherlands (4), the rest of the samples with a Danish indirect mix-ELISA (5). Both assays are based on the same combination of O-antigens, i.e. 1, 4, 5, 6, 7 and 12, and were inter-calibrated, so that results could be compared. Results were expressed in Optical Densities (OD), where samples with OD-values over 40 were considered positive. In total, 440 herds were involved in this study. In each herd, the risk factor questionnaire was applied by personal interview of the herd owner.

Statistical Method

Serological results were combined with information collected on the herd factors by questionnaire to carry out a *Salmonella* risk factor analysis. The response variable was defined as the number of sero-positive samples out of the number of samples taken per herd.

As suggested by Martin (6), a basis model was chosen, based on a priori knowledge of some important confounders, in this case the country of origin (COUNTRY) and feed related factors (7, 8), to be used for trivariable screening. Due to the problem of multicollinearity between feed related factors in this study, i.e. wet or dry feed (WETORDRY), home-mixed or purchased (HOMEMIX), pelleted or non-pelleted (PELLET), only the variable PELLET was chosen to be included in the basis model, since it explained more of the variation present in the data than the other two variables together and there was full agreement on the definition of this variable among participating countries.

Subsequently, all candidate explanatory variables were screened by logistic regression models, using the GENMOD Procedure (9), where each candidate variable was added one at a time to the basis model, containing COUNTRY and PELLET. During this process, the parameter estimate for PELLET was monitored for large deviations between the basis model and the model, including the candidate explanatory variable, as a means of assessing confounding already

at this stage of the analysis.

All variables with a level of significance less than 0.25 (P<0.25) were then included into a full model, which was subsequently reduced by backwards elimination, up to the point where the remaining parameter estimates had a significance level of approximately 0.15 or less. Two-factor interactions were created between all remaining variables and included in the model. Afterwards, backwards elimination was continued to reduce the number of non-significant interaction terms.

The serological results in this study were clustered within each herd, as herds were clustered within countries. Overdispersion in the data, because of clustering at the herd level, was adjusted for by using the P-scale option in the GENMOD procedure. This option calculates a scale parameter from the square root of the Pearson's Chi-square statistic divided by the degrees of freedom, which adjusts the standard errors of the parameter estimates appropriately. The purpose of including the variable COUNTRY in the model was to adjust for the confounding effect of between country variation, not as a potential risk factor. Given that only few countries are involved in this study, the variable COUNTRY was included in the model as a fixed effect.

Results

During the screening of candidate explanatory variables, the deviation of the parameter estimate for PELLET (Beta = -1.4886) remained fairly stable with a range of 0.51.

A total of 11 explanatory variables, including those from the basis model, were considered for analysis in the full model. Twelve herds were excluded from the final analysis because of missing data for one or more variables in the final model. The final model is based on results from 20.775 serological tests from 388 pig herds.

In a previous 'end-model', not presented here, a significant interaction between COUNTRY and PELLET was found (P=0.0088). In an attempt to explain this finding, it was further investigated, whether the effect of PELLET actually differed between countries. It was suspected that the use of wet feed (WETORDRY) was more common in certain countries than others and that the use of wet feed was generally inversely correlated to the use of pelleted feed.

Therefore, a new variable (FEED) was created out of the 4 combinations of the variables WETORDRY and PELLET. Replacing PELLET with FEED in the model effectively eliminated the interaction with COUNTRY (P=0.2803), indicating that the previously found interaction was falsely introduced because of multicollinearity between the two feed related factors and that the effect of feed did not depend on the country of origin.

Since herd selection procedures differed between countries and the variable COUNTRY was only included in the model to correct for between-country variation, the parameter estimates for COUNTRY are without meaning and therefore not presented here.

Table 1 shows the herd factors that were found to be associated with sero-positivity for *Salmonella*. The parameter estimates were re-calculated to Odds Ratios and the corresponding P-values were derived with Likelihood Ratio tests, which are argued to be more accurate than Wald's test (9).

Table 1. Herd factors associated with sero-positivity for *Salmonella*, at a sample cut-off OD>40.

Variable	Parameter	Odds Ratio	P-value
FEED	Pelleted and Dry (PD	8.2	0.0001
	Pelleted and Wet (PW)*	10.4	0.0523
	Non-pelleted and Dry (ND)	4.2	0.0094
	Non-pelleted and Wet (NW)	1	
BATCH	Continuous production (N)	2.0	0.0323
	Batch production (Y)	1	
WHEY	No use of whey (N)	5.6	0.0866
	Use of whey (Y)	1	

* Combination only observed in two herds.
Scale parameter 3.64

In Table 1 can be seen that in this study, a blood sample from a herd feeding Pelleted, Dry feed (PD) is 8 times more likely to test positive than a sample from a herd feeding Non-pelleted, Wet feed (NW).

From the comparison between the combined factors Non-pelleted, Dry (ND) and Non-peleted, Wet (NW), where Non-pelleted is kept constant, it was found that there is an increased risk of feeding Dry feed over Wet feed with a factor of approximately 4. The single effect of pelleted feed is best demonstrated in a contrast between Non-pelleted, Dry (ND) and Pelleted, Dry (PD) (see Table 2), since the estimated parameter for the combination of pelleted and wet feed (PW) was found in two herds only, and should therefore be interpreted with caution.

Pigs in herds with a continuous production system, were found to have a two fold increase in risk for sero-positivity compared to herds with batch production (all in-all out) in this study. In other words, samples from continuous flow herds are twice as likely to be found positive as samples from batch production herds.

In the final model, the variable WHEY was not found significant at the 0.05-level, but at the 0.10 level (P=0.0866). At this level of significance, herds using whey are 5 times less likely to yield *Salmonella* sero-positive results.

By means of Likelihood Ratio Contrast statements and changing the reference parameter in the model, a significant difference was found between two levels of FEED, namely between ND and PD (P=0.0245). This indicates a significant decrease in the risk for sero-positivity when feeding non-pelleted feed as opposed to pelleted feed (see Table 2).

Table 2. Direct comparison between levels of the combined variable FEED, by means of Likelihood Ratio Contrast statements.

Contrast statement	Odds Ratio	P-value
Non-pelleted and Dry (ND)	0.5	0.0245
Pelleted and Dry (PD)	1	
Pelleted and Wet (PW)	1.3	0.8003
Pelleted and Dry (PD)	1	

No significant difference was shown between PW and PD because of too few observations of PW. However, the protective effect of wet feed over dry feed can be clearly seen in Table 1, when comparing ND with NW (OR=4.2).

The factors in this study which were considered for model building, but for which no significant association with sero-positivity for *Salmonella* could be shown included: adding acid to either feed or water, adding antibiotic growth promoters to feed, feeding ad libitum versus restricted, the number of supplier herds, the number of pigs present in the herd, open pen separations versus closed, and slaughter weight of finishers.

Discussion

One of the main objectives of veterinary epidemiology is to identify and quantify risk factors, influencing animal health and animal production. Though *Salmonella* seldom causes major clinical outbreaks in pigs, it is generally regarded as an important zoonosis, and therefore of great public concern. Since pigs can be infected with *Salmonella* without any obvious symptoms of disease, there is a need to identify herds with infected animals to avoid spread of the infection throughout the production chain.

A well documented method for detecting sub-clinical *Salmonella* infections in pigs is by analysing blood samples for the presence of antibodies against *Salmonella* (e.g. 4, 5, 10, 11). Despite the inability to distinguish between current and past infections, results are believed to indicate if pigs have been exposed to *Salmonella* during production, thereby reflecting the bacteriological *Salmonella* status of the herd of origin.

The Danish mix-ELISA has been applied with success in the Danish *Salmonella* Surveillance Programme (12) over the last few years. However, the Danish test was developed specifically to detect the majority of *Salmonella* serotypes prevalent in Denmark. This may lead to sensitivity and/or specificity problems when the test is applied in other countries, having each their specific *Salmonella* spectra or even other pathogens which may give cross-reactions. This phenomenon was investigated extensively on sera originating from Sweden (13), where serological results did not correlate to bacteriological findings. Dahl (10) found the correlation between serological and bacteriological results to be higher at OD>40 than at OD>10, commonly referred to as the 'experimental cut-off' (5). In order to minimise the number of false-positive samples, a cut-off value of OD>40 was used here, which is also applied in the Danish *Salmonella* Surveillance Programme.

To make full use of the serological information available in the data set, and thereby increasing the power of the study, the analysis was performed on the proportion of seropositive samples per herd, rather than dichotomizing the outcome at herd-level into positive or negative. In doing so, the selection of a herd-level cut-off (e.g. a herd is considered positive when two or more samples are positive) was avoided.

The herd selection process for this study differed between participating countries. However, it is argued here that, since no active measures were taken or changes made related to the herd *Salmonella* status, results can be combined in the risk factor study. Though, this means that the parameter estimates for COUNTRY can not be used to compare Odds Ratios between countries.

The efforts to control *Salmonella* infection in pigs should be a combination of minimising or preventing exposure to *Salmonella* and maximising pig resistance. Batch production has been hypothesised to reduce the possibility for exposure to a number of pathogens and is commonly accepted as an important aspect in Good Farming Practice. Producing pigs according to the all in- all out principle, should help prevent cross-contamination between batches. It also allows for cleaning and disinfection between batches, which may obstruct the formation of a 'house-strain cyclus' of repeated re-infection from the environment. However, few studies have been able to demonstrate any benefit from batch production over continuous production. The findings presented here, support the general belief in a beneficiary effect of batch production in avoiding and controlling *Salmonella* contamination.

Feed can play an important role in the exposure of pigs to *Salmonella* on a herd-level, but also has an impact on the physiology of the individual animal. Increasing pig resistance may be obtained through a change in feeding strategy. The odds for testing a blood sample positive were found to be 8 times higher in herds feeding pelleted, dry feed compared to herds feeding non-pelleted, wet feed.

It is clear that almost always water is added to non-pelleted feed to make wet feed and that pelleted feed almost exclusively is dry. However, the other two combinations, especially non-pelleted, dry feed, are also found. Therefore it

was necesarry to create a new variable (FEED) with 4 levels for all combinations, to deal with multicollinearity.

Though the two feed related factors investigated in this study were highly correlated, their effect could be estimated separately by testing the appropriate contrasts between levels of the new variable. The positive association between pelleted feed and sero-positivity demonstrated here, has been shown in a number of independent studies (7, 8) and has been the cause of some debate recently. Whether the increased risk of feeding pelleted feed is due to heat treatment, the pelleting process or the coarseness of the feed is not clear, but the matter has been investigated in a recently completed study by Joergensen et al. (8).

The decrease in risk of using wet feed over dry feed has also been shown before (7, 14). Several explanations have been offered, including that a lowering of the pH during a natural fermentation process in wet feed, inhibits the growth of *Salmonella* (15). It is not unlikely that the indication of a protective effect of whey found in this study (OR= 5.6, P=0.0866), is based on the same principle. Often, the use of whey is related to wet feeding systems. It is therefore included here because the protective effect of organic acids in whey and fermented by-products against (sub-clinical) *Salmonella* infections have been discussed in a number of papers and are considered as a possible intervention method at the herd-level (14, 15, 16).

The results from this study support earlier observations that feeding pelleted feed is associated with an increased risk of sero-positivity for *Salmonella* compared to feeding non-pelleted feed and that wet feed and the use of whey are associated with a reduced risk for sero-positivity. Moreover, it was shown that the odds of testing sero-positive are twice as high in herds with a continuous production system compared to herds with batch production.

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